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Morphological and phylogenetic clarification of *Peziza arvernensis, P. pseudovesiculosa, P. pseudosylvestris*, and *P. domiciliana*

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Abstract—The concepts of the morphologically similar species *Peziza arvernensis*, *P. pseudovesiculosa*, *P. pseudosylvestris*, and *P. domiciliana* are revisited in light of new morphological and molecular data from freshly collected and herbarium specimens. Phylogenetic inference based on ITS nrDNA sequences suggests that *P. arvernensis*, *P. pseudovesiculosa*, and *P. pseudosylvestris* are monophyletic and that *P. domiciliana* represents an independent taxon.

Key words—*Pezizales*, taxonomy

Introduction

The distinction between *Peziza arvernensis* (subject of previous phylogenetic studies by Norman & Egger 1999 and Hansen et al. 2001, 2002), *P. pseudovesiculosa* [≡ *Aleuria amplissima* Boud.], *P. pseudosylvestris* [≡ *Galactinia pseudosylvestris*], and *P. domiciliana* is controversial because macro- and microscopic morphological characters seem insufficient to unambiguously define these taxa. Moreover, despite our several requests for samples of *P. pseudovesiculosa* and *P. pseudosylvestris* to institutional and private herbaria, we were able to obtain and examine only a few collections under these names.

Furthermore, *P. pseudovesiculosa* and *P. pseudosylvestris* are inadequately described (Gamundí 1975; Donadini 1977, 1978, 1979; Gamundí & Giaiotti 1998; Baiano et al. 2000; Moyne 2008). The two species seem to differ from

P. arvernensis primarily in the thickness of the layers of the excipulum, while all other characters overlap among the three species. Some authors (Hohmeyer 1986, Baiano et al. 2000) considered them to represent separate species, based on the thickness of the excipulum layers and a few other macroscopical and ecological features. The abundant observations reported by Donadini (1977, 1978, 1979, 1981) do not contribute significantly to a better delimitation of these taxa, because the characters he used are not decisive.

In the present work, we obtained new morphological and molecular data to clarify the phylogenetic position of these taxa, including: (1) Boudier's original material of *Aleuria amplissima* (PC 0084256, PC 0084257, PC 0084258), analyzed also by Donadini (1977) when he proposed the replacement name *P. pseudovesiculosa;* (2) original specimens described as *Galactinia pseudosylvestris* by Gamundí (1975); and (3) modern Italian specimens representing *P. domiciliana* Cooke.

Materials & methods

Material studied

The study was carried out on both fresh and dried samples (including holotypes) from the following herbaria—AMB, HMA, K, PC, MCVE, SIENA, TAAM, UPS—as well as from several private collections. The thirteen herbarium specimens selected for DNA extraction are listed in Table 1.

Table 1. Newly sequenced specimens of *Aleuria*, *Galactinia*, and *Peziza* used in phylogenetic analysis

Species	Country	Collector & herbarium no.	ITS sequence
A. amplissima	France	E. Boudier PC 0084257	KC832902
	France	E. Boudier PC 0084258	KC832903
G. pseudosylvestris	Argentina	I. Gamundí (UPS) F-570657	KC832900
	Chile	I. Gamundí (UPS) F-570665	KC832899
P. arvernensis	Belgium	herb. D. Tanchaud DT 26051301	KP125489
	Italy	herb. F. Padovan FP 84	KC832897
	Italy	herb. A. Lantieri AL 0406055; K(M) 134792	KC832896
P. domiciliana	Italy	herb. G. Medardi AL 11030266; MCVE 16472	KC832906
	Italy	herb. G. Medardi GM 27058844	KC832907
	Italy	herb. F. Padovan FP 2014	KC832904
P. pseudovesiculosa	Italy	herb. G. Medardi GM 16100447	KC832901
	Italy	herb. G. Medardi GM 22099945	KC832898
	Italy	herb. G. Medardi GM 28100346	KC832905

Specimens examined:

Peziza arvernensis: **BELGIUM**: Vallonia, Fôret de Soignes Brabant Wallon, on the ground in a deciduous wood, 26.05.2013, leg. & det. D. Tanchaud (DT 26051301; GenBank KP125489). **DENMARK**: Zealand, Stenholdtsvang, Grib Skov, on sandy soil, 14.08.1965, leg. P.M. Petersen, det. H. Dissing (TAAM 187941). **ENGLAND**: Herefordshire, Ross-on-Wye, on sandy soil, 19.06.1977, leg. & det. W.D. Graddon (K(M) 42798); Surrey, Sheepleas, West Horsley, on the ground in a deciduous wood with *Fagus*, 12.10.1981 (K(M) 50729); West Sussex, Madehurst, on the ground under deciduous trees, 2.07.1978, leg. D.A. Reid (K(M) 50718). **ESTONIA**: Tartu Co., along the road to Tallinn, on the ground in *Fagus* litter, 13.05.2007, leg. L. Tedersoo, det. K. Hansen (TAAM 192338). **ITALY**: Abruzzo, Prato di Camposecco, near woody residue, 10.10.1984 (HMA 3754); Teramo, Intermesoli, Fonte Novello, Pietracamela, on the ground in a *Fagus* wood, 20.06.2004 (HMA 6216); Basilicata, Potenza, Picerno, Monti li Foi, on the ground in a *Fagus* wood near fallen wood, 24.10.1998, leg. & det. G. Medardi (MCVE 14131); Calabria, Reggio Calabria, Molochio, on the ground in a mixed deciduous wood (mostly *Fagus*), 11.11.1988, leg. & det. F. Padovan (FP 159); Gambarie, near woody residue in a *Fagus* wood, 18.10.2003, leg. & det. G. Medardi (MCVE 16614); near woody residue in a *Fagus* wood, 6.06.2010, leg. & det. B. Recchia (BR 7386); Campania, Salerno, Camalonga, 11.06.2008, leg. G. Ruocco, det. L. Pecoraro & P. Leonardi (SIENA 6546); Emilia-Romagna, Reggio Emilia, Bergogno, Casina, on the ground in a *Fagus* wood, 28.04.1991, leg. & det. G. Simonini (MCVE 7282); Lazio, Terni, Trevi del Lazio, 20.07.1986 (HMA 4128); Roma, Cervara di Roma, 30.07.1986 (HMA 4127, as *Peziza repanda*); Piemonte, Vercelli, Alagna Valsesia, near woody residue in a mixed deciduous wood, 25.08.1995, leg. & det. P.G. Jamoni (PGJ 931, as *Peziza pseudosylvestris*); Sicily, Catania, Maletto, Parco dell'Etna, near woody residue in a *Fagus* wood, 2.05.2004, leg. & det. A. Lantieri (AL); Messina, Cesarò, Parco dei Nebrodi, on the ground in a mixed deciduous wood, 4.06.2005, leg. & det. A. Lantieri (K(M) 134792, AL 0406055; GenBank KC832896); Trentino-Alto Adige, Belluno, Monte Palmina, Canale d'Agordo, on the ground, 24.08.1996, leg. & det. E. Bizio (MCVE 10690); Tuscany, Pisa, Calambrone, on sandy soil near *Cistus*, *Juniperus* and *Ammophila* in marine environment, 26.04.1996, leg. & det. G. Medardi (MCVE 11261); Veneto, Belluno, Campon, Bosco del Cansiglio, on the ground near *Fagus*, 25.07.1988, leg. & det. F. Padovan (FP 84; GenBank KC832897); Padola, on the ground, 21.08.2012, leg. & det. A. Testoni (GM 21081216); Tambre, Pian Osteria, Loc. Canaie, under deciduous, 7.07.2001, leg. G. Robich, det. E. Campo (MCVE 15980); Vicenza, Recoaro, Malga Chempele, under deciduous, 5.06.2004, leg. & det. G. Medardi (MCVE 16797). **SCOTLAND**: Highland, Inner Hebrides, Isle of Skye, Armadale Castle, on sandy soil, 24.05.1982, leg. & det. R.W.G. Dennis (K(M) 50726); on sandy soil, 27.09.1983, leg. & det. R.W.G. Dennis (K(M) 50731); on sandy soil, 15.05.1984, leg. & det. R.W.G. Dennis (K(M) 50730); Perth and Kinross, Birks of Aberfeldy, under deciduous, 2.08.2010, leg. J. & D. Bailey, det. B.M. Spooner (K(M) 166578). **SWEDEN**: Närke, Lanna, on the ground, 12.07.1981, leg. & det. L. Södeberg (UPS F-640159).

Peziza domiciliana: **ITALY**: Abruzzo, Aquila, Chiarino, near woody residue of *Fagus*, 24.05.2000 (HMA 12); Provvidenza, 2.10.2000 (HMA 519); Calabria, Reggio Calabria, Gambarie, near woody residue of *Fagus*, 7.11.1988, leg. & det. A. Para (AMB 000236, as *Peziza micropus*); on the ground under *Fagus*, 9.12.2000, leg. & det. G. Medardi (MCVE 15807); Lombardia, Brescia, Ceto, on sandy soil under mixed deciduous trees, 27.05.1988, leg. & det. G. Medardi (GM 27058844; GenBank KC832907); Veneto, Belluno, near woody residue of *Fagus*, 15.04.1993, leg. & det. F. Padovan (FP 2014; GenBank KC832904), Padova, Monselice, on the ground under deciduous trees, 5.03.1997, leg. & det. G. Medardi (MCVE 12412); **Sicily**, Siracusa, R.N.O. Vendicari, on sandy soil in a mixed wood, 11.03.2002, leg. & det. G. Medardi (MCVE 16472, AL 11030266; GenBank KC832906).

Peziza pseudovesiculosa: ITALY: **Emilia-Romagna**, Reggio Emilia, Marola, on the ground under *Fagus*, 23.05.1997, leg. G. Baiano, det. D. Garofoli (MCVE 12816); **Lombardia**, Brescia, Manerba del Garda, 22.09.1999, leg. & det. G. Medardi (GM 22099945; GenBank KC832898); Calvagese, on the ground under *Castanea*, 4.11.2000, leg. & det. G. Medardi (MCVE 15497); on the ground in a mixed deciduous wood (mostly *Carpinus* and *Alnus*), also near burnt residue, 28.10.2003, leg. & det. G. Medardi (GM 28100346; GenBank KC832905); Puegnago del Garda, under mixed deciduous trees,16.10.2004, leg. & det. G. Medardi (GM 16100447; GenBank KC832901); **Piemonte**, Alessandria, Vignole Borbera, fraz. Variano Superiore, under deciduous trees, 22.04.2009, leg. & det. M. Carbone (MCVE 24022); **Tuscany**, Siena, Riserva di Cornocchia, 26.05.2010, leg. H. Danielli, L. Pecoraro & E. Salerni, det. L. Pecoraro & E. Salerni (SIENA 6547); **Veneto**, Belluno, Falcade, Caviola, near *Fagus*, 15.08.1984, leg. & det. E. Bizio (EB 15088407).

Aleuria amplissima: FRANCE: **Paris**, Île-de-France, Val d'Oise, Bois de Beauchamp et Forêt l'Isle-Adam, on sandy soil in the forest, s.d., leg. & det. E. Boudier (PC 0084256, holotype); on sandy soil in the forest, 04.1889, leg. & det. E. Boudier (PC 0084258; GenBank KC832903); on sandy soil in the forest, 04.1902, leg. & det. E. Boudier (PC 0084257; GenBank KC832902).

Galactinia pseudosylvestris: ARGENTINA: **Tierra del Fuego**, Ushuaia, on the ground in a deforested area, 21.01.1940, leg. & det. I. Gamundí (UPS F–570656 [containing 6 collections]; UPS F–570657 [containing 4 collections]; GenBank KC832900). CHILE: **Tierra del Fuego**, Magallanes y Antárctica Chilena, Rio Rubens, on the ground in a *Nothofagus pumilio* forest, 11.01.1941, leg. & det. I. Gamundí (UPS F–570666 [containing 10 collections]); Canal Whiteside, Puerto Yartou, on the ground in a *Nothofagus pumilio* forest, 12.02.1941, leg. & det. I. Gamundí (UPS F–570665 [containing 4 collections]; GenBank KC832899).

Morphological studies

Measurements and descriptions of microscopic characters were made using material mounted in water, rehydrated when necessary with 5% KOH. Melzer's reagent and Cotton blue in lactic acid were also used. An Optika BK 1301 microscope with 40× or 100× (immersion oil) objectives was used to study and photograph the morphological features. Spore dimensions were calculated by measuring 50 mature spores.

DNA isolation, PCR, and sequencing techniques

Total DNA was extracted from dried specimens (Table 1) following a CTAB–based procedure. 600 µl of 2X CTAB–buffer was added to a small piece of ascoma and blended with the aid of a micropestle. It was then incubated at 65°C for 15 min; a volume of chloroform: isoamylalcohol (24:1) was added; and the mixture was gently shaken to emulsify both phases and centrifuged at 13,000 g.

One volume of isopropanol was added to the recovered supernatant, and the mixture was centrifuged again at 13,000 g for 15 min. The resulting pellet was washed in 70% ethanol, centrifuged for 2 min, dried, and finally eluted in 200 µl ddH_2O.

PCR amplifications each comprised a 45 µl working volume to which 1–2 µl of template DNA was added. Primer pairs ITS1F-ITS4, ITS1F-ITS2, or ITS3-ITS4 White et al. (1990), and Gardes & Bruns (1993) were used to amplify nrDNA the ITS1 and IT2 (or ITS1+2) regions. Amplification protocols were: 95°C initiation for 5 min; 35 cycles at 95°C for 45 s, 54°C for 30 s, and 72°C for 45 s; and a final step at 72°C for 10 min. PCR products were checked in 1% agarose gel prior to purification and sequencing with primers ITS1F or ITS4.

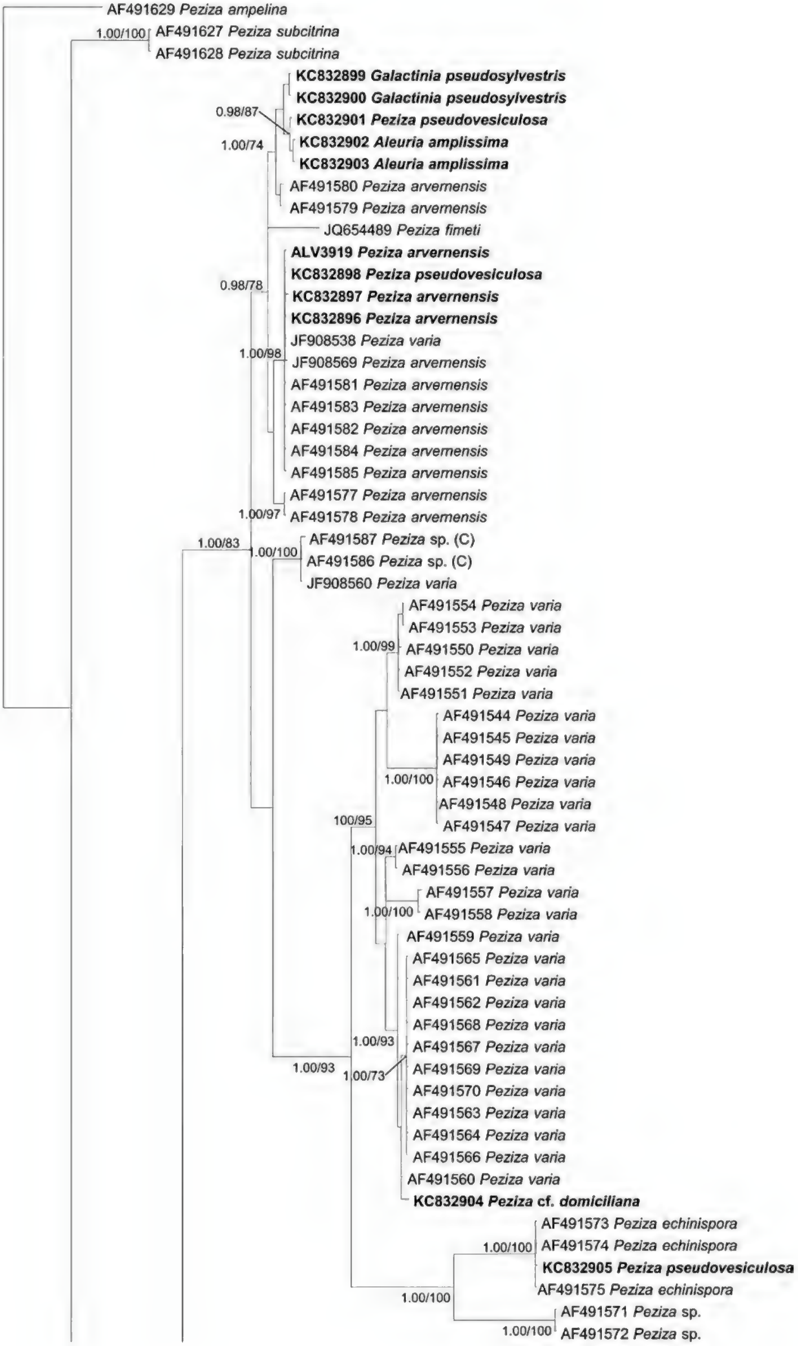
Chromatograms were visually checked in MEGA5 (Tamura et al. 2011) to detect ambiguous nucleotides and sequencing problems. Consensus sequences were placed in GenBank (Table 1). Newly generated sequences and their closest relatives in public databases were incorporated into the alignment produced by Hansen et al. (2002) stored in TreeBase (M1221), and only slight manual modifications were needed to accommodate insertions. Final analyses included 902 positions, of which 316 were parsimony-informative. A maximum parsimony (MP) search was performed in PAUP*4.0b10 (Swofford 2001) using standard conditions (2000 bootstrap replications, TBR swapping algorithm, 50 sequence additions per replicate, MULTREES not in effect). 50% majority-rule consensus tree bootstrap values were annotated in the best-scoring phylogram evaluated after a maximum-likelihood score analysis of all trees recovered. Finally, a Bayesian analysis was performed in MrBayes 3.1 (Ronquist & Huelsenbeck 2003), where the best-scoring model in MrModeltest 2.3 (Nylander 2004) was simulated. Six chains were employed with temperature set to 0.2, sampling every 100th generation until convergence parameters were met after 2,150,000 generations.

Results & discussion

The analyses were congruent with each other in the suggested topology (Fig. 1) and similar to the phylogenetic relationships already reported for this group (Hansen et al. 2002, Medardi et al. 2012).

Our results imply *P. pseudovesiculosa* [≡ *A. amplissima* Boud.] and *P. pseudosylvestris* as closely related to *P. arvernensis* within a monophyletic clade, sister to those formed by *P. varia* (Hedw.) Alb. & Schwein. and by *P. fimeti* (Fuckel) E.C. Hansen and related taxa. Within this monophyletic clade, the ambiguity of the taxonomic relationships among the different lineages could be subject to conflicting interpretations. We choose to consider them all as synonyms of *P. arvernensis* (the oldest available name) because of their insufficient genetic differences, the absence of diagnostic morphological features, and the similarly high intraspecific variability seen in the sister species *P. varia*. This synonymy can be summarised as:

Peziza arvernensis Roze & Boud., Bull. Soc. Bot. Fr. 26 (Suppl.): LXXVI. 1879.
= *Peziza pseudosylvestris* (Gamundí) Alessio, Micol. Ital. 4(2): 21. 1975.
  ≡ *Galactinia pseudosylvestris* Gamundí, Fl. Cript. Tierra del Fuego 10(3): 37. 1975.
= *Peziza pseudovesiculosa* Donadini, Bull. Soc. Linn. Provence 30: 60. 1977.
  ≡ *Aleuria amplissima* Boud., Hist. Class. Discom. Eur.: 44. 1907, nom. illeg. (non *A. amplissima* (Fr.) Pat. 1904).
  ≡ *Peziza amplissima* Sacc. & Traverso, Syll. Fung. 20: 309. 1911, nom. illeg. (non *P. amplissima* Fr. 1849).
  ≡ *Galactinia amplissima* Svrček, Česká Mykol. 16: 110. 1962.


AF491629 *Peziza ampelina*
1.00/100
AF491627 *Peziza subcitrina*
AF491628 *Peziza subcitrina*
KC832899 *Galactinia pseudosylvestris*
KC832900 *Galactinia pseudosylvestris*
0.98/87
KC832901 *Peziza pseudovesiculosa*
1.00/74
KC832902 *Aleuria amplissima*
KC832903 *Aleuria amplissima*
AF491580 *Peziza arvernensis*
AF491579 *Peziza arvernensis*
JQ654489 *Peziza fimeti*
ALV3919 *Peziza arvernensis*
KC832898 *Peziza pseudovesiculosa*
0.98/78
KC832897 *Peziza arvernensis*
KC832896 *Peziza arvernensis*
JF908538 *Peziza varia*
1.00/98
JF908569 *Peziza arvernensis*
AF491581 *Peziza arvernensis*
AF491583 *Peziza arvernensis*
AF491582 *Peziza arvernensis*
AF491584 *Peziza arvernensis*
AF491585 *Peziza arvernensis*
AF491577 *Peziza arvernensis*
1.00/97
AF491578 *Peziza arvernensis*
1.00/83
AF491587 *Peziza* sp. (C)
1.00/100
AF491586 *Peziza* sp. (C)
JF908560 *Peziza varia*
AF491554 *Peziza varia*
AF491553 *Peziza varia*
1.00/99
AF491550 *Peziza varia*
AF491552 *Peziza varia*
AF491551 *Peziza varia*
AF491544 *Peziza varia*
AF491545 *Peziza varia*
AF491549 *Peziza varia*
1.00/100
AF491546 *Peziza varia*
AF491548 *Peziza varia*
100/95
AF491547 *Peziza varia*
1.00/94
AF491555 *Peziza varia*
AF491556 *Peziza varia*
AF491557 *Peziza varia*
1.00/100
AF491558 *Peziza varia*
AF491559 *Peziza varia*
AF491565 *Peziza varia*
AF491561 *Peziza varia*
AF491562 *Peziza varia*
AF491568 *Peziza varia*
1.00/93
AF491567 *Peziza varia*
1.00/93
1.00/73
AF491569 *Peziza varia*
AF491570 *Peziza varia*
AF491563 *Peziza varia*
AF491564 *Peziza varia*
AF491566 *Peziza varia*
AF491560 *Peziza varia*
KC832904 *Peziza* cf. *domiciliana*
AF491573 *Peziza echinispora*
1.00/100
AF491574 *Peziza echinispora*
KC832905 *Peziza pseudovesiculosa*
AF491575 *Peziza echinispora*
1.00/100
AF491571 *Peziza* sp.
1.00/100
AF491572 *Peziza* sp.

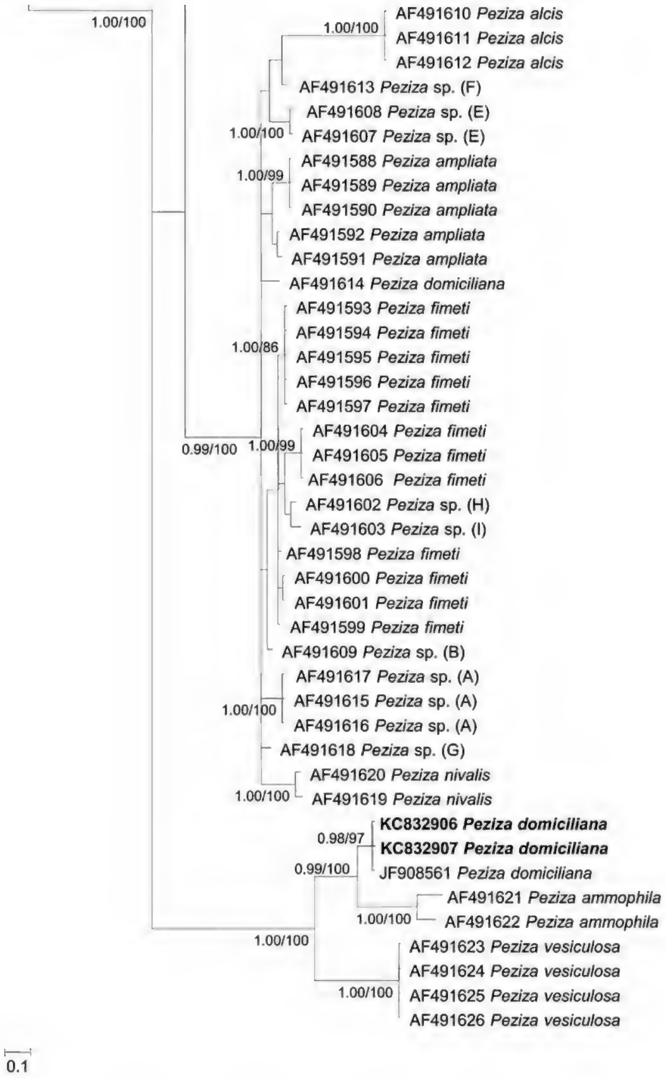


FIG. 1. Consensus ITS phylogram of *Peziza* s. str. obtained in MrBayes 3.1. Bayesian posterior probabilities and maximum parsimony bootstrap proportions are presented for nodes significantly supported by at least one of these analyses. Our new sequences are set in bold font.

TABLES 2–5 summarise morphological data for *P. arvernensis*, *P. pseudovesiculosa*, *P. pseudosylvestris*, and *P. domiciliana*, and make comparisons between data from the literature and from our study.

TABLE 2. *Peziza arvernensis:* comparison of cited and new morphological data

CHARACTER	ROZE & BOUDIER (1879)	CURRENT STUDY
APOTHECIUM	Regularly cup-shaped to undulate, fragile, rather thick, subsessile	Often rather deep cup-shaped, tending to open in age, fragile, rather thick, sessile
HYMENIUM	Ochraceous-ferruginous with olivaceous tints	± Pale brown, brown-beige, sometimes pale tobacco, at times with reddish-violaceous or fawn tints
RECEPTACLE	Ochraceous-ferruginous with olivaceous tints, slightly furfuraceous. Base with white tomentum	Concolorous with the hymenium or greyish ochraceous-fawn with rust tints, tending to lighten when dried. Smooth to slightly furfuraceous
ASCOSPORES	16–18 × 8–9 µm, finely warted	16–19(–20) × 9–10(–11) µm, finely warted
ASCUS	(not reported)	270–280 × 15–16 µm
PARAPHYSES	Clavate above, septate, slightly coloured, with several oil droplets	Clavate, 6–8 µm above; subcylindrical, 3–4 µm below; septate
EXCIPULUM	(not reported)	SH: text. globulosa-angularis, cells 13–24 µm diam. ME: 3-layered (Upper & Lower layers: text. globulosa, cells 35–90 µm diam; Intermediate: text. intricata, hyphae 5–10 µm diam EE: text. intricata, hyphae 5–10 µm diam, mixed with globose cells 15–20 µm diam
HABITAT	Ground, also between mosses on one thatch	Ground, also sandy soil; marine to mountain environment, under deciduous trees (*Fagus*, *Quercus*), at times also near burnt residue or on woody remnants

SH = Subhymenium; ME = Medullary excipulum; EE = Ectal excipulum

The microscopical analyses of *Aleuria amplissima* revealed spores measuring 14–16(–18) × 8–9 µm, finely warted, with multiple small internal guttules and asci measuring 270–300 × 14–16 µm. We observed an ectal excipulum composed of septate hyphae 9–12 µm in diam. arranged in a textura intricata, more or less perpendicular to the hymenium and parallel to one another, instead of the textura globulosa-angularis reported by Donadini (1977, 1978, 1979) and Baiano et al. (2000). These characters are remarkably similar to those observed in several *P. arvernensis* collections: our observations of more or less finely warted spores of 16–19(–20) × 9–10(–11) µm and asci 270–280 × 15–16 µm) agreed with those by Korf (1985). We also noticed a similar excipular structure and spore measurements in original material of *A. amplissima* (PC 0084256, PC 0084257, PC 0084258) and in *G. pseudosylvestris*.

Since the type material of *G. pseudosylvestris* in LPS was not available for study, other specimens collected and identified by Gamundí were analyzed

TABLE 3. *Peziza pseudovesiculosa*: comparison of cited and new morphological data

CHARACTER	DONADINI (1977)	CURRENT STUDY
APOTHECIUM	Cup-shaped, rarely opened, fragile, thick, sessile or nearly so	Cup-shaped, tending to open in age, fragile, ± thick, sessile or nearly so
HYMENIUM	Ochraceous to ochraceous-brown	± Pale brown, brown, with some reddish tints
RECEPTACLE	Paler than the hymenium, furfuraceous	± Concolorous with the hymenium, furfuraceous
ASCOSPORES	15–18 × 8–10 µm, finely warted	16–19 × 9–10(–11) µm, finely warted
ASCUS	240–270 × 14–16 µm	270–280 × 15–16 µm
PARAPHYSES	Cylindrical, 3–4 µm below; clavate, 5–10 µm above; septate	Subcylindrical, 3–4 µm below; clavate, 6–8 µm above; septate
EXCIPULUM	SH: text. globulosa-angularis, cells 7–15 × 15–20 µm ME: 3-layered (Upper: text. globulosa-angularis, cells 15–90(–130) µm diam.; Intermediate: text. intricata & porrecta, hyphae 6–12 µm diam; Lower: text. globulosa, cells 30–80 µm diam.) EE: text. globulosa, cells 15–25 µm diam. with protruding hyphae 7–17 µm diam.	SH: text. globulosa-angularis, cells 10–25 µm diam. ME: 3-layered (Upper: text. globulosa, cells 30–80 µm diam; Intermediate: text. intricata, hyphae 5–10 µm diam; Lower: as first layer) EE: text. intricata, hyphae 5–10 µm diam, mixed with globose cells 12–17 µm diam.
HABITAT	Humus, woody remnants, burn residue	Humus, woody remnants, burn residue

SH = Subhymenium; ME = Medullary excipulum; EE = Ectal excipulum

(UPS-F570656, UPS-F570665, UPS-F570666, UPS-F570657). These samples showed spores measuring (15–)16–19(–19.5) × 8.5–11 µm, with punctiform warts ca. 0.5 × 0.5 µm and asci ≤280–320 × 12–14 µm. Gamundí (1975) argued that *G. pseudosylvestris* could be distinguished from *P. arvernensis* by its thicker excipulum, especially the upper and lower layers of the medullar excipulum. Development of more or less wide cells in the excipulum and of moniliform or thread-like paraphyses seems, however, to be correlated with a more or less high level of humidity, as stated by Kullman (1995) and Hansen et al. (2002). Nonetheless, we observed an average thickness ca. 1500–1600 µm in all rehydrated samples, in line with the measurements observed in "typical" *P. arvernensis*. We conclude that this species does not represent an independent lineage at the species level, but should be considered a synonym of *P. arvernensis*.

Most authors mentioned that *P. arvernensis* sensu stricto is typically associated with *Fagus sylvatica* L. (fruiting on soil, on litter, or near woody

TABLE 4. *Peziza pseudosylvestris*: comparison of cited and new morphological data

CHARACTER	GAMUNDI 1975; DONADINI 1978	CURRENT STUDY
APOTHECIUM	Cup-shaped, then more opened, subsessile to stalked	—
HYMENIUM	Pale ochraceous, pale hazel, ochraceous to olivaceous	—
RECEPTACLE	Concolorous to paler than the hymenium, furfuraceous	—
ASCOSPORES	14.1–18.2 (–19.2) × 7.2–9.6 µm, finely warted	(15–)16–19 (–19.5) × 8.5–11 µm, finely warted
ASCUS	240–355 × 9.6–14.4 µm	280–320 × 12–14 µm
PARAPHYSES	Thread-shaped, 2.4–4.3 µm large, septate, containing ochraceous granules	Subcylindrical, 3–4 µm below; clavate, 6–7 µm above; septate
EXCIPULUM	SH: text. globulosa, cells 8.4–15.6 µm diam. ME: 3-layered (Upper: text. globulosa, cells 50–120 µm diam; Intermediate: text. intricata, hyphae 4.8–9.6 µm diam; Lower: text. globulosa, cells 30–96 µm diam.) EE: text. intricata, hyphae 6–9.6 µm diam	SH: text. globulosa-angularis, cells ≤20 µm diam. ME: 3-layered (Upper: text. globulosa-angularis, cells up to 80 µm diam; Intermediate: text. intricata, hyphae 8–10 µm large; Lower: as first layer) EE: text. intricata, hyphae ≤5 µm diam, mixed with globose cells 15-20 µm diam.
HABITAT	Ground, near woody residuals of *Nothofagus*	—

SH = Subhymenium; ME = Medullary excipulum; EE = Ectal excipulum

remnants), but our observations show the habitat of *P. arvernensis* (both sensu stricto and sensu lato) to be quite variable: in addition to *Nothofagus*, some specimens have been collected under *Castanea* (MCVE 15497), *Carpinus*, *Alnus*, and other mixed deciduous trees in a floodplain environment (GM 28100346); *Quercus ilex* L. (AL 0406055); typical Mediterranean vegetation near *Cistus*, *Juniperus*, and *Ammophila* in a marine retro-dunal environment (MCVE 11261); and on ground with burn residues (MCVE 15497).

Peziza arvernensis sensu lato may also be confused with *P. domiciliana*, which has very similar features: a light umber-brown hymenial surface, paler (whitish-ochraceous to sub-concolorous) outer surface, spores measuring 15–17 (–20) × 7–9(–10) µm that are both smooth and finely warted even in the same apothecium when ejected from the asci, a similar flesh architecture, and a substrate of soil, loam, or decaying wood. Sequences of two *P. domiciliana* collections (GM 27058844, AL 11030266) seem to match a sequence of another *P. domiciliana* stored in GenBank (JF908561; Garbellotto et al., unpubl.) that represents a lineage distinct from *P. arvernensis* (FIG. 1), thus supporting

TABLE 5. *Peziza domiciliana*: comparison of cited and new morphological data

CHARACTER	COOKE 1877	CURRENT STUDY
APOTHECIUM	Quite regularly cup-shaped, then cochleate and contorted, fragile	Cup-shaped, tending to open in age, fragile, rather thick, sessile or nearly so
HYMENIUM	Hues ranging from white to amethystine violet; sometimes rose-tinged	Amber-brown, honey coloured
RECEPTACLE	Concolorous	Paler than the hymenium, whitish-ochraceous
ASCOSPORES	11–13 × 6–7 µm, ornamentation not described	15–17(–20) × 7–9(–10) µm, smooth to finely warted
ASCUS	Not reported	270–300 × 13–15 µm
PARAPHYSES	Not reported	Subcylindrical 2--3 µm below, clavate 5--6 µm above, septate
EXCIPULUM	Not reported	SH: text. globulosa-angularis, cells ≤10–15 µm diam. ME: 3-layers: Upper & lower: text. globulosa, cells ≤50 µm diam; Intermediate: text. intricata, hyphae 5–10 µm diam EE: text. intricata, hyphae ≤5–10 µm diam
HABITAT	In a garden	Sandy or clay soil, loam, sawdust or other woody residue: also in cellars or caves

SH = Subhymenium; ME = Medullary excipulum; EE = Ectal excipulum

recognition of *P. domiciliana* as an independent species in accordance with Hansen et al. (2002). However, it should be noted that the sample of *P. domiciliana* sequenced by Hansen et al. (2002; AF491614) seems different from JF908561 and those sequenced in the present work and more closely related to the lineage formed by *Peziza ampliata* Pers., *P. fimeti*, and *Peziza alcis* Harmaja.

Hansen et al. (2002) noted that both *P. domiciliana* and *P. arvernensis* could be confused with *P. varia*. However, the spores in *P. varia* were observed by Medardi et al. (2012) as slightly smaller (14–16(–17.5) × 9–11(–12) µm), and the ornamentation in *P. varia*, very difficult to observe with a conventional light microscope in either surface or outline view, is readily visible in *P. domiciliana* and *P. arvernensis*. Nevertheless, one of our collections determined morphologically as *P. domiciliana* (FP 2014) fell instead in the clade of *P. varia*.

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